

The search for antithrombotic therapy

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The Search for Antithrombotic Therapy

H.C. Hemker

There is no disease in the western world that demands a higher toll in death and disability than thrombotic disease. Coronary infarction, stroke, circulation problems in the legs, many cases of hypertension and scores of other diseases are all members of one big family: atherosclerotic vessel disease. All these diseases start by microthrombosis at the inside of the vessel wall and they usually end by either massive occlusive thrombosis or bleeding.

If you would think thrombosis therefore will be the main concern of medical research you are mistaken! Most people, even most doctors, even indeed most cardiologists, are barely aware of the important role of thrombosis as a mechanism of disease. This is readily seen if we have a look at the research efforts in different fields. If for every patient that dies from circulatory disease in the Netherlands fl. 500.- is spent on research, then fl. 3000.- is paid for every cancer patient and fl. 15000.- for every AIDS patient.

For years and years even the research efforts in the field of circulation have touched the subject of thrombosis only lightly. Cardiologists have done marvellous work in plumbing and electricity, that is to say in haemodynamics and in electrophysiology. This has been very useful in mending the damage brought about by the obstruction of vessels but it left aside the cause of the obstruction. Actually it is not until very recently, with the advent of thrombolytic therapy, that most cardiologists became aware of the role of thrombosis in the diseases they so frequently see.

I will not dwell upon the causes of this strange situation. It is a fact however, that every layman will tell you that unlimited cell growth is the cause of cancer but many doctors will be insecure about the role of vessel disease in the mortality of our population.

Some of you, who have refrained from eating eggs and fat meat for a number of years will get uneasy now. Has it not been demonstrated, they think, that cigarette smoking, cholesterol, diabetes, lack of physical exercise etc. are the cause of atherosclerosis rather than thrombosis?

Indeed, epidemiological research has defined a number of risk factors that correlate with the occurrence of coronary infarction. In a sense one can say that smoking causes coronary infarction and other circulatory problems. People who do not smoke will get less than half the trouble of those who do smoke. If I maintain that thrombosis is at the basis of atherosclerotic heart and vessel disease I mean to say that it is the common pathogenetic in these diseases.

It is the old but always disturbing question of correlation and cause. There is an established correlation between malaria and the bad air of the marshlands. So good indeed that it served to give the disease its name. There is a good correlation between unhappy love and tuberculous consumption, or at least it was thought to be observed. Still it was not until the falciparum malaria and its vector the mosquito were discovered that a rational combat of malaria could begin. It was not until tuberculosis was recognized to be of bacterial origin that a rational medical attack on this disease was possible. In an analogous way we can say that smoking indeed does cause vessel disease, but that it does so via thrombosis.

It is high time that we should recognize that thrombosis is the mechanism underlying vessel disease and that research on thrombosis is the only way to attack this scourge. This being said you will not be amazed that the rest of my talk is devoted to thrombosis.

What is thrombosis? It is the formation of a kind of blood clot at the inside of a vessel. Please note that I deliberately say *a kind of* blood clot because even though it is a solid mass originating from liquid blood, it is not the same as coagulated blood such as we may observe it when blood clots outside the body.

Three components interact when a thrombus forms: a) the vessel wall, b) the formed elements of the blood, and c) the blood plasma. Without going into any detail we can say that any thrombosis starts by a lesion of the vessel wall. This brings the blood in contact with broken cells. Such cells contain a particular lipoprotein called *thromboplastin*. This thromboplastin interacts with a plasma protein called Factor VII and in that way starts the process of thrombin formation. If the vessel wall lesion is slightly more pronounced so that the cells of the inner lining not only break but give way so as to expose the underlying tissue, then the thrombocytes will stick to the collagen of that tissue. The simultaneous presence of collagen and thrombin activates the platelets and makes them help in generating more thrombin. This causes more platelets to adhere to the wounded vessel wall and it will cause fibrinogen from the blood plasma to transform into fibrin fibers. In this way a solid mass comes into being. If the wound in the vessel wall is an exterior trauma then this solid mass is called the haemostatic plug and is a life saving necessity that prevents us from bleeding to death. If the vessel wall lesion is brought about by atherosclerosis then a thrombus forms. Thrombus and haemostatic plug can be regarded as two sides of the same coin. In the one case it is life-saving, in the other life-threatening. This immediately shows one of the problems of thrombosis therapy: it should be prevent thrombosis but leave haemostasis intact. The problem is not unlike that of antibiotics, that should kill bacteria but leave the cells of the body intact, or of tumor chemotherapy, that should attack malignant cells but not normal ones.

Now who will find such a therapeutic for thrombosis? What type of research will lead to new efficient antithrombotics?

The three components of the thrombosis mechanism, the platelet, the vessel wall and the plasma interact very closely indeed but the

people who study them usually do not. In the laboratories of haemostasis and thrombosis research it is easy to recognize different specialists: the coagulationist, who studies the clotting of plasma, the plateletologist who studies the platelets, and the wallflower who studies the vessel wall. They hardly ever are encountered all three in the same lab, however.

The coagulationists are the oldest branch. Modern research on coagulation started in the forties with the work of Owren, Koller and MacFarlane. The study of the platelet began to flower in the early sixties, mainly because of the pioneering efforts of Gustav Born and Jacques Caen. The vessel wall is the most recent focus of attention. One may mention the studies on prostanoids by Vane and the culture of endothelial cells by Jaffe.

In defining a strategy for further action one first has to decide what approach of these three is the most promising. The answer is of course that we should try an integrated approach, but integration has to start somewhere. In trying to decide upon a strategy it is perhaps best to start from what has been achieved. Already in the first half of this century the coagulationists gave us anticoagulation in two different varieties: Oral anticoagulation and heparin therapy. Both of them have been proven to be very efficient antithrombotic treatments but both of them have major drawbacks too. They are difficult to apply well, and if not managed perfectly they are either ineffective or dangerous because of the bleeding complications that they can cause.

The study of platelets, in more than 20 years of research has essentially left us empty handed as far as therapeutics are concerned! Indeed, there is only one antiplatelet drug that has unequivocally been shown to be effective in preventing coronary infarction: good old Aspirin. Many other drugs are at the best as active as Aspirin is, even though they inhibit platelet function in a number of different ways.

The study of the vessel wall brought a Nobelprice to Vane and lots of interesting data at that, but in the way of therapeutics not much has been achieved as yet. Notably the therapeutic manipulation of the prostanoids has not resulted in a recognized novel antithrombotic

therapy. Aspirin indeed does inhibit cyclooxygenase and thus thromboxane synthesis but then it has not been proven that it is this action of Aspirin that is responsible for its antithrombotic effect.

If we are to plan a serious attack on thrombotic disease I would be in favour of basing ourselves of these data and recognize that the only effective antithrombotic drugs are anticoagulants. This means drugs that inhibit the coagulation enzyme thrombin. Both oral anticoagulants and heparin have a variety of effects on other things than thrombin, so you might reason that it may be one of the other activities that is actually antithrombotic. I cannot exclude this possibility but in defence of my standpoint I can say that heparin on the one hand and oral anticoagulants on the other are so different that the only effect that we know them to have in common is their effect on blood coagulation. We are left of course with Aspirin to which I shall come back later.

Oral anticoagulants and heparins are also very different in the way of their action on the coagulation mechanism. Oral anticoagulants are antagonists of vitamin K. Vitamin K is necessary for the synthesis of certain coagulation factors. When there is no vitamin K then there will be a lack of coagulation factors, among which prothrombin, and the thrombin formation in clotting blood will be diminished. In fact I discovered in 1963 that under these circumstances prothrombin is present but in a modified and unactive form. Later this led to the discovery of the mode of action of vitamin K and the role of certain modified aminoacid residues in blood. This helped in establishing the correct way of executing the treatment but it does not change the view that, under oral anticoagulation for all practical purposes prothrombin is diminished. Recent results from our laboratory suggest that only the diminution of prothrombin influences the clotting process in oral anticoagulation.

Heparin acts very differently from oral anticoagulants. Recently we could show that the action of classical heparin in plasma is based on one phenomenon only: the fact that it makes thrombin disappear more quickly. The only thing that oral anticoagulants and heparin have in common therefore is their action on the

amount of thrombin that is formed in clotting plasma. We recently did a thorough study on the newly developed low molecular weight heparins. These promise to have many advantages compared to classical heparin but they still act on thrombin or its formation.

This is the basis for my conviction that thrombin is the final common pathway of all types of thrombosis. The attack on thrombosis should therefore be an attack on thrombin.

This may not seem very revolutionary to the non-specialist but you must be aware that in the last twenty years the leading paradigm has been that the most killing type of thrombosis, arterial thrombosis, is essentially a platelet disease. Therefore, the search for new antithrombotics in the last 20 years has been primarily a search for antiplatelet drugs and I propose that this may have been the wrong approach.

What may have been the reasons for the prime importance attributed to platelet aggregation? I can see two: one is the concept of the essential difference between arterial and venous thrombosis, the other is the innate opportunism of the research scientist who is fascinated by a new technique.

Every pathologist can tell you about the difference between the white and the red thrombus and usually he will oppose them as if the Russian revolution still raged in our bloodstreams. The white thrombus consists mainly of blood platelets sticking to each other and to the collagen of the vessel wall. They are held together by fibrin threads but apart from that there is few evidence of blood coagulation. The white thrombus is the one found in the arterial system, the plug that causes infarction and stroke. The red thrombus in the classical description is hardly different from clotted blood. It consists of a fibrin mesh in which the cellular elements of the blood are caught. Erythrocytes are abundant, hence its red colour. Would it not be logical then to attack the white -, that is to say the platelet thrombus, with antiplatelet drugs and the red one with anticoagulant therapy?

More important still: Anticoagulant therapy proved very effective against red, venous thrombi but for a long time it was much more

difficult to prevent arterial thrombosis in that way. It thus seemed logical to attack the platelet first.

As to the second reason, you can imagine how enthusiastic the researchers became when Born announced his simple method for the study of platelet aggregation. They immediately seized the occasion to have a direct look at the process that made platelet thrombi. Antiplatelet drugs became synonymous with aggregation inhibitors and lots of them were found. It came as an unpleasant surprise that many of these drugs that perfectly inhibited platelet aggregation in practice still would not make very good antithrombotics. In my opinion this is because aggregation is not the adequate model for the arterial thrombus.

I feel that the microscopical structure of the white thrombus as seen by the pathologist, together with the fascination of the discovery of Born have made the scientific world jump to the conclusion that aggregation would be the *in vitro* representation of arterial thrombosis and that good aggregation inhibitors would make good antithrombotics. This is not true. Sometimes they make reasonable antithrombotics, like Aspirin, sometimes perfect aggregation inhibitors make bad antithrombotics, like PGE₁.

On the other hand it appeared that oral anticoagulation, when applied steadily and deeply was not as bad as that in preventing arterial thrombosis. This has been conclusively shown by the trials conducted by Loeliger and his colleagues in Leiden. Also in the earliest stages of the formation of a haemostatic plug as well as in the earliest stages of the formation of an arterial thrombus, fibrin can be observed almost immediately. From this one can deduct that thrombin must be there already at the moment that platelets aggregate to any appreciable extent. In fact, it is probably thrombin that makes the platelets aggregate. That thrombin plays such an important role in phenomena that, morphologically, seem to be dominated by platelets needs not surprise us. Thrombin is known to be the most potent physiological platelet activating agent. It is active at concentrations that are one or more orders of magnitude higher than ADP, adrenalin and every other possible platelet activator.

This last observation points to the direction in which we will have to search for new antithrombotics. It is rather the cooperation between platelets and the thrombin forming mechanism that should be our point of attack than either the platelet or the clotting system alone. Some of our recent experimental results illustrate this point.

We investigated the development of thrombin in platelet rich plasma and compared it to platelet poor plasma. In PPP in the course of 20 min hardly any thrombin develops. In PRP however, after 10–12 min there is a sudden burst of thrombin formation. This is because very small amounts of thrombin activate the platelets. The activated platelets then, in their turn, trigger an outburst of thrombin formation. This immediately demonstrates that it is silly to think that thrombin formation and platelet activation are isolated phenomena. One should recognize that they are tightly coupled in a positive feedback loop. As soon as platelets and plasma are both present they interact. This becomes even more evident if we try to imitate the effect of tissue wounding by adding tiny amounts of thromboplastin to the system. Added in such a high dilution that in PPP no increase of thrombin formation can be seen, it will still have a marked effect in PRP. It will cause the burst of thrombin formation to occur about 4 min earlier than in absence of thromboplastin. This means that minute cell damage will promote thrombin generation in PRP even though it will have no influence on PPP. At higher concentrations of thromboplastin the difference between PRP and PPP become less important, so that in the usual laboratory tests, like the PT, the effect of platelets will be hardly observable.

We see that the effects of small amounts of tissue thromboplastin are only observed if both plasma and platelets are present. I think that coagulation in presence of low concentrations of thromboplastin in platelet rich plasma may be a more realistic model of the *in vivo* situation than isolated platelet or isolated plasma alone, were it only because the three components that are due to encounter in *in vivo* thrombus formation are present here.

We investigated the effect of antithrombotic drugs in this system. It appeared that the three recognized therapeutics of thrombosis all

postponed the explosion of thrombin in PRP whereas other substances like PGE 1, that can completely inhibit aggregation but that have hardly any effect of thrombosis, do not influence the cooperation between platelets and plasma.

These results make us surmise that this cooperative effect might be a good indicator of antithrombotic actions, a good screening test for antithrombotic drugs.

Now there are two possibilities, either this is true or it is not. If it is true than we have obtained a comparatively easy way to find new antithrombotics, no matter whether they act primarily on platelets, like Aspirin, or on the clotting system, like the various heparins and heparin-like drugs. It is equally possible, however, that our approach after all will not lead to the development of new drugs. Then the investigation will not lead to the development of new drugs. Then the investigation of the novel cooperative effect between blood coagulation and platelets will anyhow increase our knowledge and thus may indirectly contribute to the development of better thrombosis treatment.

For the next few years it seems that the daily practice of thrombosis treatment will have to do with carefully standardized oral anticoagulation and with low molecular weight heparins. In the near future other thrombin generation inhibitors may be expected to be developed. Antiplatelet drugs that specifically inhibit the procoagulant activity of blood platelets may be among the more promising ones.

Zusammenfassung

Thrombotische Erkrankungen fordern in der westlichen Welt den höchsten Zoll an Toten und führen zu verbreiteter Invalidität. Mikrothrombose an der Gefäßinnenwand ist Anfang und Ursache fast aller Infarkte, Apoplexien, Durchblutungsstörungen, Hochdruckerkrankungen. Und doch: weder nach Umfang noch Intensität wird der entsprechende Forschungsaufwand dieser Tatsache gerecht. Wir setzen in Holland pro Patient bei Kreislauferkrankungen nur 500 Gulden an Forschungsmitteln ein, bei Krebs aber 3000 und bei AIDS sogar 15000.

In der Tat: Das Wissen über die Thrombose ist wenig verbreitet, und erst die thrombolytische Therapie hat auch die Kardiologen auf ihre Bedeutung aufmerksam werden lassen. Immer noch hält der Laie – und gewiß nicht ohne Grund! – Rauchen, hohen Cholesterinspiegel, Diabetes, Bewegungsmangel für die Ursachen von Arteriosklerose; er denkt nicht primär an Thrombose. Für mich aber steht fest: Thrombose liegt allen arteriosklerotischen Herz- und Gefäßerkrankungen zugrunde. Die Erforschung der Thrombose ist damit der Weg zur Bekämpfung dieser Krankheiten.

Was ist Thrombose? Bei ihrer Entstehung wirken drei Faktoren zusammen: Gefäßwand, Blutplättchen, Blutplasma. Gefäßwandschädigungen setzen über Thromboplastin und Plasmaprotein (F VII) einen Prozeß in Gang, der bei äußeren Verletzungen zur Bildung des lebensrettenden, bei Arteriosklerose zum Entstehen des lebensgefährdenden hämostatischen Pfropfs führt. Damit wird ein zentrales Problem der Therapie deutlich: sie muß der Thrombose vorbeugen, ohne die Blutgerinnung auszuschalten.

Die drei Komponenten Gefäßwand, Blutplättchen, Blutplasma wirken in fataler Perfektion zusammen, viel besser als die Spezialisten, die sie untersuchen: die Koagulationsforscher, die Blutplättchenspezialisten, die Gefäßwandexperten. Bessere Integration ist wichtig. Die Antigerinnungsmittel sind wirksam, aber auch gefährlich. Aspirin hat sich gut gegen Plättchenkonglomerate bewährt. Bei der Gefäßwandforschung registrieren wir noch keinen therapeutischen Durchbruch.

Meine Forschungen und Erfahrungen haben mich zu der Überzeugung geführt: Thrombin ist der Schlüssel zur Thrombose. Wer Thrombose bekämpfen will, muß das Thrombin bekämpfen. Das sage ich vor allem im Blick darauf, daß man in den letzten 20 Jahren beim tödlichsten Typ der Thrombose, der arteriellen, die Konglomeration der Blutplättchen für entscheidend hielt. Das war – meine ich – ein Fehlansatz, der wohl mit auf der konventionellen Unterscheidung zwischen „weißer“ und „roter“ Thrombose beruhte.

Aber Plättchenaggregation – Borns Forschungen waren hier bahnbrechend – ist wohl

doch nicht das Entscheidende bei arterieller Thrombose. Es stimmt nicht, daß wir im Aggregationsprozeß der Plättchen, wie wir ihn in vitro konstruieren, den Ablauf einer arteriellen Thrombose in vivo sehen. Auch gute Aggregationshemmer schützen keineswegs zuverlässig vor Thrombose.

Wahrscheinlich führt nämlich das Thrombin zur Plättchenkonglomeration. Thrombin ist der stärkste Wirkungsfaktor bei der Aktivierung der Blutplättchen. Hier müssen wir ansetzen. Unsere Versuche haben gezeigt, wie eng die Plättchendichte im Plasma und das schubartige Auftreten von Thrombin zusammenhängen. Auch kleinste Gefäßwandverletzungen wirken übrighen steigernd in dieser Richtung.

Wenn unsere Beobachtungen und die aus ihnen folgenden therapeutischen Ansätze stimmen, wird es möglich sein, neue antithrombotische Mittel zu entwickeln, die vor allem auf das Thrombin zielen. Natürlich schließt ihr Einsatz die Anwendung und Weiterentwicklung von sorgfältig abgestimmten gerinnungshemmenden Mitteln, Heparinen mit niederem Molekulargewicht und Antikoagulantien im Plättchenbereich nicht aus.

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Professor Hemker wurde 1985 mit dem Ernst-Jung-Preis für Medizin ausgezeichnet. Er erhielt den Preis für seine richtungsweisenden Forschungen über die Blutgerinnung, die neue Erkenntnisse und wesentliche Fortschritte in der Thrombosetherapie erbracht haben. Professor Hemker lehrt und arbeitet an der Universität von Limburg in Maastricht – Department für Biochemie.

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